Reprint from GENETICS, Vol. 46, No. 11, November, 1961. *Printed in U.S.A.*

A STABILIZER OF ANTIGENIC PHASES IN SALMONELLA ABORTUS-EQUI[1]

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Received May 30, 1961

IN diphasic strains of Salmonella, the flagellar antigen type oscillates between phase-1 and phase-2. The phenomenon has been known as phase variation. Its genetic mechanism was studied in detail by LEDERBERG and IINO (1956). The frequency of phase variation is generally as high as 10^{-5} to 10^{-3} per cell per division (STOCKER 1949); but a much lower frequency of the alternation has been observed in certain Salmonella serotypes, e.g. in *S. abortus-equi* and *S. paratyphi A* (BRUNER and EDWARDS 1941; EDWARDS and BRUNER 1939). In the present paper, a genetic factor is reported which controls the low frequency of phase variation in *S. abortus-equi*.

MATERIALS AND METHODS

As a stable phase strain, *S. abortus-equi* CDC-26 was used. The strain is stable in phase-2, *enx* type, which is determined by H_2^{enx}. The presence of the suppressed phase-1 antigen type determinant H_1^a in a phase-2 clone was demonstrated by transduction of H_1^a as well as H_2^{enx}, to various recipients (LEDERBERG and EDWARDS 1953) and by rare spontaneous change to the stable phase-1 type (LEDERBERG, personal communication). The cells of CDC-26 move rather slowly in both phases being even slower in phase-1 than in phase-2. Strain TM2 of *S. typhimurium* was used for comparison as a diphasic type. TM2 expresses *i* type in phase-1 and *1.2* type in phase-2 (designated by *i*: *1.2*). The change from phase-2 to phase-1 in TM2 occurs at the rate of 3×10^{-4} per cell per division in a broth culture, and at about one fourth of that from phase-1 to phase-2.

The phase-1 antigen type of a stable phase-2 strain was determined by linked transduction of Fla_1^+ and H_1 from that strain to *S. paratyphi B* SW666. SW666 is a strain without flagella originated from phase-1 (*b* type) monophasic *S. paratyphi B*, by mutation of Fla_1^+ to Fla_1^-, which is linked to H_1. The hidden phase-1 antigen type of the stable phase-2 strain is detected in some recombinants of the transduction.

The general procedures of cultivation, transduction and selection of serotypic recombinants were according to the methods of LEDERBERG and IINO (1956).

[1] Contribution from the National Institute of Genetics, Japan. No. 388. This work was supported by a research grant from the National Institute of Allergy and Infectious Diseases (E-2872), Public Health Service, U.S.A.

Phage PLT22 was used as the vector in transduction (ZINDER and LEDERBERG 1952). The selection of alternative phases was carried out by the semisolid nutrient gelatin agar (NGA) tube method described by EDWARDS and EWING (1955). Antigen types were determined by slide agglutination.

RESULTS AND CONCLUSIONS

Phase stability of S. abortus-equi: A stock culture of CDC-26 was streaked on a nutrient agar plate. After 24 hour incubation at 37°, each of 20 isolated colonies was transferred to broth and cultivated overnight on a rotator; the culture reached about a concentration of 2×10^8 cells per ml. Two tenths ml of each broth culture was then dropped in a NGA tube supplemented with anti-*enx* serum. The amount of anti-*enx* serum was so adjusted as to prevent the moving of *enx*-type cells but not that of the cells of other antigen types (EDWARDS and EWING 1955). A series of phase-2 cultures of TM2 was prepared in the same way and 0.2 ml samples were likewise dropped into an NGA tube supplemented with anti-*1.2* serum. In parallel, another 0.2 ml was dropped from each of both CDC-26 and TM2 cultures into a plain NGA tube. They were incubated at 37° C and observed day by day. In all of the tubes, CDC-26/plain, TM2/plain and TM2/anti-*1.2* serum, grown cells moved into NGA and formed swarms within two days after they were inoculated. The antigen types of the cells of those swarms were *enx* in CDC-26/plain, *1.2* in TM2/plain and *i* in TM2/anti-*1.2* serum tubes. In CDC-26/anti-*enx* tubes, however, no swarms were detected even after five days prolonged cultivation. The experiments were repeated five times, and the same results were obtained each time: no swarms were produced in any CDC-26/anti-*enx* tubes but they were produced from all others.

To test the possibility that nonmotile type cells are produced in phase-1, broth cultures of CDC-26 were spread on NGA plates and the segregation of two colonial types, compact and swarm, was examined by the same method as the test of Ah_1^- (IINO 1961). Regular segregation as in Ah_1^- strains was not observed in CDC-26.

Thus, the phases of *S. abortus-equi* CDC-26 are exceedingly stable in contrast to those of a diphasic strain *S. typhimurium* TM2. The stable type phase and the unstable type are easily identified by NGA tube cultures.

Spontaneous changes of phase have occurred from *enx* to *a* in *S. abortus-equi*, but only very rarely (EDWARDS and BRUNER 1939). One *a* clone was obtained by anti-*enx* NGA selection from the *enx* types of *S. abortus-equi*, with the strain SL23 in the course of an experiment conducted for other purpose. The *a* type was as stable as the original *enx* type, and only one *enx* type swarm was detected from 160 anti-*a* NGA tube cultures.

Transductional analysis of the phase-stability determinant: Flagellar antigen types of Salmonella are determined by the H_1 and H_2 genes. One or the other of these genes is expressed in a given clone which is described as being in phase-1 and phase-2 respectively. A factor inseparable from H_2 is the phase determinant, alternating between two states: H_2 active vs. H_2-inactive-H_1 active. In order

to test whether the difference in the stability of H_2 is caused by its own intrinsic structure or by other genetic factor(s), transductions were carried out from an *enx* type culture of CDC-26 to an *i* type culture of TM2. Serotypic recombinants were isolated from NGA plates supplemented with anti-*i* and anti-*1.2* sera.

Among the recombinants isolated, four were diphasic *a:1.2* type, 42 diphasic *i:enx* and 19 monophasic *enx* (Table 1). That *i* is the hidden phase-1 of the last

TABLE 1

Transductions between S. typhimurium *TM2 and* S. abortus-equi *CDC-26. Hidden antigenic phases of diphasic strains or monophasic strains were parenthesized or bracketed respectively*

Donor	Recipient	Selection on	Recombinant Type	Recombinant No.	Transduced loci
CDC-26	TM2	anti-i, 1.2	a : 1.2	4	H_1^a
[a]: enx	i : (1.2)	NGA	i : enx	42	H_2^{enx}
			[i]: enx	19	$H_2^{enx}\ Vh_2^-$
			Total	65	
TM2	CDC-26	anti-enx	[a]: 1.2	38	$H_2^{1.2}$
(i): 1.2	[a]: enx	NGA	a : 1.2	15	$H_2^{1.2}\ Vh_2$
			Total	53	

type was demonstrated on three PLT22 sensitive clones by transduction of the H_1^i to *S. paratyphi* B. SW666. Those results show that *a* and *enx* are each transduced from the phase-2 culture of CDC-26. When *a* is transduced the resulting transduction remains diphasic, whereas when *enx* is transduced some transductional clones become monophasic. By anti-*enx* serum selection, *i* phase cultures were rarely obtained from the *enx*-monophasic transductional clones. The *i* phase cultures obtained were also monophasic. The stabilization of the inherent H_2 activity in *S. abortus-equi* CDC-26 is therefore caused by a factor which is linked to H_2.

The *a:1.2*- and *i:enx*-diphasic and *enx*-monophasic transductional types obtained are almost as motile as the recipient strain TM2, which moves into NGA media three to four times faster than CDC-26. The slow motility of CDC-26, therefore, depends principally on factors independent of H_1, H_2 and the phase stabilizer.

The reciprocal transductions, from an *i* type culture of TM2 to an *enx* type culture of CDC-26, support the above conclusion (Table 1). Among 53 transductional clones screened on anti-*enx* NGA plates, 38 were stable *a* and 15 were diphasic *a:1.2*. The hidden antigens of the *a* clones are presumed to be *1.2*. The frequencies of linked transduction of the H_2 stability controller with H_2 agree well between the reciprocal transductions (about 30 percent). The H_2- stability controller will be given a symbol Vh_2. Its allele in TM2 is Vh_2^+ and Vh_2^- in CDC-26.

DISCUSSION

The present study demonstrated that the genetic factor Vh_2, which is closely linked to H_2 but separable from it, regulates the frequency of antigenic phase

variation in Salmonella. In other words, Vh_2 controls the change of the inherent state of H_2 activity. A gene which regulates mutability of other genes has been reported in some bacterial strains (TREFFERS, SPINELLI and BELSER 1954; SKAAR 1956; MIYAKE 1960). They have been called mutator genes. In contrast to those mutator genes, the function of Vh_2 as far as we know is the specific control of H_2 activity. However, no comprehensive test has been made of mutation rates in Vh_2^+ and Vh_2^- strains. Analogues to the H_2–Vh_2 systems are perhaps found in the variegation of higher organisms (LEWIS 1950), for instance the *Ac–Ds* and *Mp* elements in corn (MCCLINTOCK 1956; BRINK and NILAN 1952) and the suppression of phenotypic effect by transposition of euchromatic genes to heterochromatic regions in Drosophila (DEMEREC 1940; BAKER 1953).

A similar type of monophasic behavior as that of *S. abortus-equi* has been reported in *S. paratyphi A* (BRUNER and EDWARDS 1941; EDWARDS, BARNES and BABCOCK 1950). *S. paratyphi A* isolated from nature is usually in *a* phase (phase-1). The *a* phase is very stable and an alternative phase, phase-2 (*1.5* phase), can be obtained only through selection by anti-*a* serum. The phase-2 culture, either isolated from nature or obtained from a phase-1 culture, is also stable and rarely reverts to phase-1. The monophasic property of *S. paratyphi A* may be caused by stabilization of H_2 as in *S. abortus-equi.*

A strain that carries Vh_2^- is highly stable in either phase, and a phase stable strain of a desired antigen type may be obtained by transduction. Consequently, Vh_2^- strains are excellent materials for the production of specific flagellar antigens (EDWARDS and EWING 1955).

SUMMARY

The frequency of antigenic phase variation in Salmonella is regulated by the genetic factor Vh_2 which is closely linked to H_2; that is, Vh_2 controls the stability of H_2 state. In a strain of *Salmonella abortus-equi*, CDC-26, an allele Vh_2^- stabilizes H_2 in its existing state, whether inactive or active, and produces phase-1 or phase-2 monophasic types respectively.

ACKNOWLEDGMENT

These experiments had been started during the author's stay at the Department of Genetics, University of Wisconsin from 1955 through 1958, where they were supported by research grants from the National Cancer Institute, United States Public Health Service, (C-2157) and the National Science Foundation. The author wishes to express his sincere gratitude to DR. J. LEDERBERG for patient guidance during the course of this investigation.

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